Spectroscopic and electrochemical studies of bilayer lipid membranes tethered to the surface of gold

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It is already well recognized that the bilayer lipid membranes tethered to the electrode surface via a hydrophilic spacer molecules (t-BLMs) can provide a novel matrix that allow for the incorporation and functional detection of integral membrane proteins under non-denaturating conditions. Moreover, these biomembrane mimetic films on the electrode surface upon the immobilization of specific biocatalysts are expected to be electronically and mechanically compatible with the current sensor technology. The unique feature of these t-BLMs is that they ensure the combined, hydrophobic/hydrophilic environment, which is a prerequisite for retaining the activity and substrate specificity of membrane proteins hosted by these membranes. While the interior of t-BLMs provides the necessary hydrophobicity, the hydrophilic spacer molecules, anchoring the bilayer to the electrode are purposefully tailored to secure the "ionic reservoir" between the lipid bilayer and the surface of the electrode. The following requirements shall be met by the t-BLMs: (a) the SSSM must be robust enough to guarantee long-term stability, and must be easily and reproducibly prepared; (b) the hydrophilic spacer must be sufficiently flexible, fluid and thick to easily accommodate in a functionally active state the hydrophilic, extrinsic portion of integral proteins, which may protrude by over 20 Å from the lipid bilayer; (c) the lipid bilayer must have a transition temperature from the gel to the liquid crystalline state lower than the ambient temperature; (d) the lipid bilayer must be sufficiently insulating to allow the characterization of the transport activity of the membrane protein by electrochemical means, without the disturbing presence of stray currents originating from “pinholes” and other defects that might provide preferential pathways for electron and ionic transfer across the lipid bilayer.

Therefore, the aim of presented work is to examine the organization and electrochemical behavior of a number of supported biomimetic membrane systems by means of spectroscopic (SERS, IRAS) and electrochemical (CV, EIS) techniques. Such structural characterization was carried out for lipid monolayers formed from dipalmitoylphosphatidylethanolamine (DPPE) and cholesteryl (Chl) moieties covalently attached via hydrophilic spacer molecules with thiol terminal groups, as well as for lipid bilayers containing the above monolayers with an adjacent outer phospholipid leaflet facing the aqueous environment. Different redox probes, varying with their hydrophilic/hydrophobic character and electron exchange kinetics were used for the evaluation of molecular integrity and passivating behavior of our films. Our results show that the electrical properties of the present tethered lipid bilayers are greatly improved with respect to a number of thiolipid|lipid bilayers anchored to gold, while retaining their fluid-like behavior. This is crucial from the point of view of signal-to-noise ratio as well as a proper fluidity/resistance balance, when using such t-BLMs for the development of sensing techniques, e.g., with incorporated electrogenic integral membrane proteins in their active state.

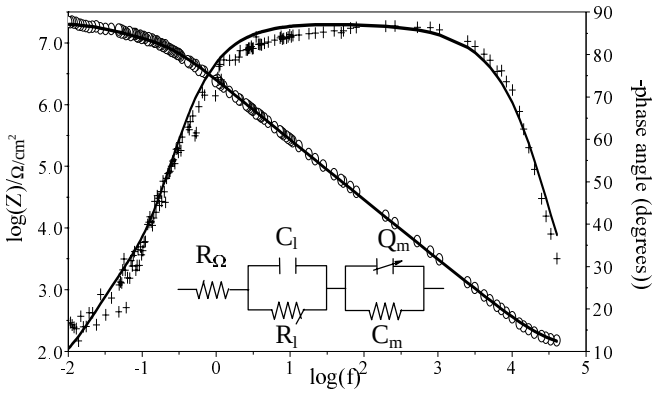

Figure 1 shows the Bode plot for the gold supported bilayer in 0.1M KCl. This system reveals its complex behavior, as the equivalent circuit describing our results contains a constant phase element. This equivalent circuit consists of the resistance, R_Ω = 111 Ω, of the supporting electrolyte, in series with a parallel arrangement of the resistance, R_l = 1.2 MΩ cm^2, and capacitance, C_l = 0.66 µF cm^{-2}, of the outer phospholipid monolayer; this is in series with a further parallel arrangement of a resistance, R_m = 1.5 MΩ cm^2 , and a constant phase element, Q (~0.52 µF/cm^2, n=0.9021), ascribable to the tethered monolayer underlying the outer phospholipid monolayer. The presence of this element reflects an imperfect organization of the thiolipid monolayer on polycrystalline gold electrode, which was confirmed also by spectroscopic studies.